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Decapod Crustacean Phylogenetics

edited by

Joel W. Martin, Keith A. Crandall, and Darryl L. Felder



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Assessing the Contribution of Molecular and Larval Morphological Characters in a Combined Phylogenetic Analysis of the Superfamily Majoidea

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ABSTRACT

Although the crab superfamily Majoidea is well recognized as a distinct grouping within the Brachyura, resolving the classification of and relationships between different majoid families has been more difficult. In this study, we combine molecular and larval morphology data in a total evidence approach to the phylogeny of the Majoidea, using sequence data from three different loci and 53 larval morphology characters from 14 genera representing 7 majoid families. We examine the relative contribution of morphological and molecular characters in resolving relationships within the superfamily Majoidea and how different alignment and tree construction methods affect tree topology. Using maximum parsimony analyses and partitioned Bremer support, we show that molecular and larval morphology partitions are congruent in combined analyses and that both types of characters contribute positively to resolution of the tree and support for major nodes. Both Bayesian analysis and direct optimization of nucleotide sequences under parsimony supported some similar relationships, including a monophyletic Oregoniidae branching at the base of the majoid tree. However, Bayesian and direct optimization trees differed in their resolution of some relationships, namely in placement of inachid and tychid species relative to the remaining majoids. Neither Bayesian nor direct optimization trees of the combined dataset supported monophyly of the majority of majoid families proposed in recent taxonomic revisions of the group, suggesting the adult morphological characters used to classify majoids into families may be incongruent with larval characters and molecular data used in this study.

1 INTRODUCTION

The crab superfamily Majoidea Samouelle, 1819, is one of the most species-rich groups of the Brachyura and is estimated to contain more than 800 species (Rice 1988) assembled into >170 different genera (Ng et al. 2008). Majoids occupy a diverse range of marine habitats worldwide (Rathbun 1925; Rice 1988), and are commonly known as “spider crabs” or “decorator crabs” because of their characteristically long legs and their distinctive behavior of attaching materials from their environment to hooked setae on their carapace to camouflage themselves against predators (Wicksten 1993). As a group, the majoids are typically thought to be one of the earliest brachyuran lineages, based on evidence from spermatozoal ultrastructure (Jamieson 1994), larval characters (Rice 1980, 1981, 1988), and molecular characters (Spears et al. 1992; Porter et al. 2005). Exact estimates of the age of this group vary; studies using model-based methods estimated that the

majoids diverged from the rest of the Brachyura ~254 MYA (Porter et al. 2005), although the earliest unequivocal majoid fossils are from the Eocene (Spears et al. 1992). The monophyly of the superfamily Majoidea is often assumed based on adult and larval morphological synapomorphies: all majoids have a terminal molt upon maturity (in contrast to other brachyurans) and only two zoeal stages (Rice 1980, 1981, 1983, 1988; but see Clark & Ng 2004). However, no study thus far has rigorously tested the monophyly of this group, and some workers have suggested inclusion of the Hymensomatidae based on affinities between hymenosomatids and inachoids (Guinot & Richer de Forges 1997; Ng et al. 2008). However, hymenosomatids differ from the majoids as they typically possess three zoeal stages and no true megalopa (Guinot & Richer de Forges 1997), and placement of the Hymensomatidae in the Majoidea is still provisional (Ng et al. 2008).

Formerly known as the family Majidae, the Majoidea were recently reclassified as a superfamily (Hendrickx 1995; Martin & Davis 2001; McLaughlin et al. 2005; Števc̆ić 2005). Diversity of the former family Majidae is very high, and recognition or treatment of the majoids as a superfamily was suggested by many early workers (Guinot 1978; Drach & Guinot 1983; Clark & Webber 1991; Števc̆ić 1994). Nevertheless, many difficulties exist in establishing different families within the Majoidea. Clark & Webber (1991) proposed recognition of family Macrocheiridae based on a reevaluation of the larval features of the genus *Macrocheira* and suggested that extant majoids be partitioned among four families: Oregoniidae, Macrocheiridae, Majidae, and Inachidae. Števc̆ić (1994) recognized six traditional families (Majinae, Mithracinae, Tychinae, Pisinae, Epialtinae, and Inachinae) and also included the Pliosominae, Planoterginae, Micromajinae, and Eurynolambrinae within Majidae. McLaughlin et al. (2005), following Griffin & Tranter (1986), recognized eight families (Epialtidae, Inachidae, Inachoididae, Majidae, Mithracidae, Pisidae, Tychidae, and Oregoniidae), the first seven of which were recognized by Martin & Davis (2001) in their recent reorganization of the Crustacea. Števc̆ić (2005) partitioned the traditionally recognized majoids into two families, the Majidae and the Inachoididae, and proposed inclusion of the families Lambrachaeidae Števc̆ić, 1994, and Paratymolidae Haswell, 1882. Most recently, Ng et al. (2008) included the hymenosomatids in the superfamily, and recognized six majoid families: Epialtidae, Hymenosomatidae, Inachidae, Inachoididae, Majidae and Oregoniidae. Here we use the traditional classification of majoids as a superfamily, split into eight recognized majoid families (Epialtidae, Inachidae, Inachoididae, Majidae, Mithracidae, Pisidae, Tychidae, and Oregoniidae; Griffin & Tranter 1986; Martin & Davis 2001; McLaughlin et al. 2005) and use molecular and morphological data to review the monophyly of (and relationships among) these groups. The majority of these familial associations follow from elevation of formerly recognized majoid subfamilies to familial status in recent taxonomic monographs (Hendrickx 1995; Martin & Davis 2001).

Several workers have examined relationships among the major groups using larval characters, primarily spination, presence and segmentation of appendages, and setation on the zoeal and megalopal stages (Kurata 1969; Rice 1980, 1988; Clark & Webber 1991; Marques & Pohle 1998; Pohle & Marques 2000; Marques & Pohle 2003). Despite differences in the conceptual framework of assessing homology in these studies (e.g., the identity of the “ancestral” and “derived” forms of majoids), they agree on some points. Kurata (1969) assumed reduction of spination and setation in larval majoids was the derived condition, and he proposed six parallel, heterogeneous lineages of majoids preceded by four different “ancestral” majoids: *Camposcia* (Inachidae), *Schizophris* (Majidae), *Maia* (Majidae), and *Pleistacantha* (Inachidae). Although he also assumed that reduction of spination and setation was the derived condition, Rice (1980, 1988) hypothesized that the Oregoniidae family retained the “ancestral” majoid larvae, and he proposed two additional lines of majoids: 1) the Inachidae, and 2) a line including the Majidae and another clade of the Pisidae and Epialtidae (formerly the Pisinae and Acanthonychinae subfamilies). Although the family Mithracidae was not considered, Rice (1988) concluded using megalopal characters that the Mithracidae was closely related to the Pisidae and Epialtidae. Phylogenies constructed from larval characters concur on some of these relationships, including a monophyletic Oregoniidae clade branching at

the base of the majoid tree (Clark & Webber 1991; Marques & Pohle 1998), and close phylogenetic relationships between the Epialtidae, Pisidae, and Mithracidae families (Pohle & Marques 2000; Marques & Pohle 2003). Marques and Pohle (2003) evaluated support for the monophyly of majoid families and found that while most majoid families were paraphyletic (with the exception of the Oregoniidae), tree lengths in which families were constrained to be monophyletic were not significantly longer than unconstrained topologies, and they concluded that larval characters could not definitively reject monophyly of majoid families. However, support for monophyly varied among different families; for example, the Oregoniidae and the Inachidae + Inachoididae groups (with the exception of *Macrocheira*) formed a clade in unconstrained analyses, while the family Pisidae never formed a clade, and tree lengths of topologies where this group was constrained to be monophyletic were significantly longer than unconstrained trees.

More recently, a molecular phylogeny of this group based on partial sequences of 16S, COI, and 28S genes has corroborated some relationships proposed from phylogenies based on larval morphology (Hultgren & Stachowicz in press). These include: 1) strong support for a monophyletic Oregoniidae; 2) poor support for monophyly of most other majoid families; and 3) close phylogenetic relationships among the families Mithracidae, Pisidae, and Epialtidae. However, molecular data could not resolve key relationships at the base of the majoid tree, namely which of three family groupings—the Inachidae, Oregoniidae, or Majidae—represented the most basally branching majoid group. This may have been due in part to difficulties with aligning portions of the DNA dataset, in particular portions of the 28S locus, suggesting it may be useful to explore if branching patterns at the base of the tree are sensitive to different alignment methods.

Prior to this study, there has been no systematic work addressing the results of simultaneous analyses of molecular and larval morphology characters to examine phylogenetic relationships in the Majoidea, despite intriguing similarities between molecular and morphological phylogenies of this group (Marques & Pohle 1998; Pohle & Marques 2003; Hultgren & Stachowicz in press) and the demonstrated utility of combining multiple sources of data in many phylogenetic studies (Baker et al. 1998; Ah Yong & O'Meally 2004). In this study, we combine molecular and larval morphological data in a 'total-evidence' approach to the phylogeny of the superfamily Majoidea, using ~1450 bp of sequence data from 3 loci (16S, COI, and 28S) and 53 larval morphology characters from 14 genera (representing 7 majoid families) to provide a more robust phylogenetic hypothesis for selected members of the Majoidea. We evaluate the relative contribution of morphological and molecular characters and explore how different alignment (static homology and dynamic homology) and tree construction methods (Bayesian and direct optimization using parsimony) affect tree topology in the superfamily Majoidea.

2 MATERIALS AND METHODS

2.1 Larval morphology

To assemble the larval morphology character database, we expanded the data matrix of Marques & Pohle (2003) by adding additional larval characters (for a total of 53) and additional taxa using species-specific descriptions of majoid larval stages. We analyzed the larval characters and codified characters of species with available DNA sequences (summarized in Appendix 1). These included *Acanthonyx petiverii* (Hiyodo et al. 1994), *Menaethius monoceros* (Gohar & Al-Kholy 1957), *Pugettia quadridens* (Kornienko & Korn 2004), *Taliepus dentatus* (Fagetti & Campodónico 1971), *Stenorhynchus seticornis* (Yang 1976; Paula & Cartaxana 1991), *Maja brachydactyla* (Clark 1986), *Micippa thalia* (Kurata 1969), *Micippa platipes* (Siddiqui 1996), *Chionoecetes japonicus* (Motoh 1976), *Hyas coarctatus alutaceus* (Christiansen 1973; Pohle 1991), *Hyas araneus* (Christiansen 1973; Pohle 1991), *Libinia dubia* (Sandifer & Van Engel 1971), *Libinia emarginata* (Johns & Lang 1977), *Pitho lherminieri* (F.P.L. Marques, unpublished data), *Herbstia condyliata* (Guerao et al. 2008), *Mithraculus sculptus* (Rhyne et al. 2006), *Mithraculus forceps* (Wilson et al.

1979), and *Microphrys bicornatus* (Gore et al. 1982). Although this represents a small taxon sample relative to the number of described majoid species, we were limited to taxa (primarily Atlantic species) for which both molecular and morphological data were available. Descriptions of character states are summarized in Appendix 2. Phylogenetic trees constructed from an earlier version of this matrix (Marques & Pohle 1998), using a non-majoid outgroup, found strong evidence for a monophyletic Oregoniidae branching at the base of the tree, similar to trees constructed from molecular data (Hultgren & Stachowicz in press). However, as larval characters coded from non-majoid crabs with >2 zoeal stages may not be homologous to characters coded from majoid crabs (which have only 2 zoeal stages), subsequent phylogenetic analyses based on larval morphology used oregoniid species as the rooting point to the remaining majoids (Marques & Pohle 2003). As larval morphology data for megalopal stages of *Heterocrypta occidentalis* were not available, we coded morphological data for this outgroup species as missing (< 5% of the total dataset for the outgroup).

2.2 Molecular data

We used sequence data from the 18 species for which we had morphological data, in addition to 7 additional congeners of those species for which we had only molecular data; in the latter case, morphological data were coded as missing (Table 1). Sampling, extraction, amplification, and sequencing methods have been described previously (Hultgren & Stachowicz in press). Briefly, we used partial sequence data from 3 loci: nuclear 28S ribosomal RNA (~600 bp), mitochondrial 16S ribosomal RNA (~430 bp), and the mitochondrial protein-coding gene cytochrome oxidase I (~580 bp, hereafter COI). Although approximately 25% of the species in the molecular data set were sequenced for only 2 out of the 3 loci, we chose to include terminals (taxa) with missing loci, as simulation studies suggested that the addition of taxa with some missing data (generally <50%) increased accuracy of the final tree (Wiens 2005, 2006). For the molecular dataset, we additionally included sequences from one outgroup species, the parthenopid crab *Heterocrypta occidentalis*.

Molecular data were initially aligned using the program MUSCLE v. 3.6 (Edgar 2004), using default parameters to align nucleotide sequences from each individual locus. Hyper variable regions were excluded from further analysis due to the ambiguity of the alignment, using the program GBlocks v.091b (Castresana 2000) and allowing all gap positions. In total, GBlocks excluded 21% of the 16S alignment, 17% of the COI alignment, and 24% of the 28S alignment. The final combined (and trimmed) molecular dataset consisted of 1478 total base pairs (BP) of sequence data. This alignment was used to test incongruence between molecular and morphological data in all analyses examining the relative contribution of molecular vs. morphological data and in Bayesian analyses of the combined molecular + morphology dataset.

2.3 Comparisons of molecular and morphological data partitions

To test whether there were significant incongruities between molecular and morphological datasets, we excluded all additional species from a genus that were not explicitly described in the larval morphology studies. Using the program PAUP ver. 4.0b10 (Swofford 2002) and the molecular alignment described above, we used the incongruence length difference (ILD) test (Farris et al. 1994) implemented under maximum parsimony (MP) to test whether molecular and morphological data were congruent.

Because molecular data often comprise a much higher proportion of characters in combined datasets relative to morphological data and may overwhelm the phylogenetic signal from morphological data (Baker et al. 1998; Wahlberg et al. 2005), we examined the relative contribution of both datasets. Using taxa with both morphology and molecular data, we examined the relative contribution of molecular and morphological characters in the combined dataset by calculating the number

Table 1. Familial associations, molecular data, and larval morphology references for different species used in the study. Familial associations are given according to the classifications of McLaughlin et al. (2005) and Ng et al. (2008).

| Species | Family | | GenBank Accession Nos. | | | | Larval morphology reference |
|--|--------------------------|-------------------------------|------------------------|--------------------|----------|---|-----------------------------------|
| | (McLaughlin et al. 2005) | (Ng et al. 2008) | 16S | COI | 28S | | |
| <i>Chionoecetes bairdi</i> (Rathbun, 1924) | Oregoniidae | Oregoniidae | AY227446 | AB21159 | — | — | |
| <i>Chionoecetes japonicus</i> (Rathbun, 1924) | | | AB188685 | AB211611 | — | — | Motoh 1976 |
| <i>Chionoecetes opilio</i> (Fabricius, 1788) | | | EU682768 | EU682832 | EU682875 | — | Christiansen 1973, |
| <i>Hyas araneus</i> (Linnaeus, 1758) | | | EU682771 | EU682834 | EU682878 | — | Pohle 1991 |
| ^a <i>Hyas coarctatus alutaceus</i> Brandt, 1851 | | | EU682774 | EU682835 | — | — | Christiansen 1973, |
| ^b <i>Stenorhynchus</i> | Inachidae | Inachidae | <i>unpublished</i> | <i>unpublished</i> | — | — | Pohle 1991 |
| ^c <i>Maja brachydactyla</i> (Balss, 1922) | Majidae | Majidae (sf. Majinae) | DQ079723 | EU000832 | DQ079799 | — | Yang 1976, Paula & Cartaxana 1991 |
| <i>Micippa thalia</i> (Herbst, 1803) | Mithracidae | Majidae (sf. Mithracidae) | EU682780 | EU682844 | EU682883 | — | Clark 1986 |
| <i>Micippa platipes</i> (Ruppell 1830) | | | EU682779 | — | EU682884 | — | Siddiqui 1996 |
| <i>Microphrys bicornatus</i> (Latreille, 1825) | | | EU682781 | EU682843 | EU682885 | — | Gore et al. 1982 |
| <i>Mithraculus forceps</i> (Milne-Edwards, 1875) | | | EU682782 | EU682840 | EU682886 | — | Wilson et al. 1979 |
| <i>Mithraculus sculptus</i> (Lamarck, 1818) | | | EU682784 | EU682841 | EU682887 | — | Rhyne et al. 2006 |
| <i>Pitho lherminieri</i> (Schramm, 1867) | Tychidae | Epiplatidae (sf. Tychinae) | EU682789 | EU682839 | EU682891 | — | Marques et al. unpublished data |
| <i>Acanthonyx petiverii</i> (Milne-Edwards, 1834) | Epiplatidae | Epiplatidae (sf. Epiplatinae) | EU682803 | EU682855 | EU682903 | — | Hiyodo et al. 1994 |
| <i>Menaethius monoceros</i> (Latreille, 1825) | | | EU682805 | EU682857 | EU682904 | — | Gohar & Al-Kholy 1957 |
| <i>Pugettia dalli</i> (Rathbun, 1893) | | | EU682810 | EU682860 | EU682907 | — | — |
| <i>Pugettia gracilis</i> (Dana, 1851) | | | EU682813 | EU682863 | EU682909 | — | — |
| <i>Pugettia minor</i> (Ortmann, 1893) | | | EU682815 | — | EU682910 | — | — |
| <i>Pugettia producta</i> (Randall, 1840) | | | EU682817 | EU682865 | EU682912 | — | — |

Table 1. continued.

| Species | Family | | GenBank Accession Nos. | | | | Larval morphology reference |
|---|--------------------------|---------------------------|------------------------|----------|----------|--|-----------------------------|
| | (McLaughlin et al. 2005) | (Ng et al. 2008) | 16S | COI | 28S | | |
| <i>Pugetia quadridens</i> (deHaan, 1850) | | | EU682824 | EU682869 | EU682916 | | Kornienko & Korn 2004 |
| <i>Pugetia richii</i> (Dana, 1851) | | | EU682826 | EU682871 | EU682917 | | – |
| <i>Taitepus dentatus</i> (Milne-Edwards) | | | EU682827 | EU682872 | EU682918 | | Fagetti & Campodomico 1971 |
| <i>Herbstia condyliata</i> (Fabricius, 1787) | Pisidae | Epiplatidae (sf. Pisinae) | EU682790 | EU682845 | – | | Guerao et al. 2008 |
| <i>Libinia dubia</i> (H. Milne Edwards, 1834) | | | EU682794 | EU682847 | EU682894 | | Sandifer & Van Engel 1971 |
| <i>Libinia emarginata</i> (Leach, 1815) | | | EU682796 | EU682849 | EU682896 | | Johns & Lang 1977 |
| <i>Libinia mexicana</i> (Rathbun, 1892) | | | EU682797 | – | EU682897 | | – |
| <i>Heterocrypta occidentalis</i> (Dana, 1854) | | Parthenopidae | EU682767 | EU682829 | EU682874 | | – |

^a Molecular data from *Hyas coarctatus* (Leach, 1815), morphological data from *Hyas coarctatus alutaceus* (Brandt, 1851).

^b Molecular data came from *Stenorhynchus lanceolatus* (Brullé, 1837) (16S) and *Stenorhynchus seticornis* (Herbst, 1788) (28S); morphological data from *Stenorhynchus seticornis*.

^c Molecular data for 16S and 28S came from GenBank *Maja squinado* specimen (Porter et al. 2005); subsequent revisions of this genus and comparison of sequence data with several *Maja* species (Sotelo et al. 2008) indicate the GenBank specimen is likely *Maja brachydactyla*.

of phylogenetically informative characters (PI) for each partition using PAUP*. We also calculated partitioned Bremer support (PBS) (Baker & Desalle 1997; Baker et al. 1998) for each data partition at each node using the program TreeRot v.2 (Sorenson 1999).

2.4 *Bayesian phylogenetic analysis*

Bayesian trees were run using the combined molecular + morphological dataset (with the molecular alignment produced by MUSCLE and GBlocks as described above) using the program MrBayes v3.1.2. (Ronquist & Hulsenbeck 2003). Prior to Bayesian analyses, we used the program Modeltest v.3.7 (Posada & Crandall 1998) to select the appropriate model of molecular evolution for each of the individual molecular loci (i.e., the model that best fit the data) using the Akaike Information Criterion (Posada & Buckley 2004) and allowing MrBayes to estimate parameters for each partition substitution model. Bayesian posterior probabilities (BPP) were obtained for different clades by performing three independent runs with four Markov chains (consisting of 2,000,000 generations sampled every 100 generations). When the log-likelihood scores were found to stabilize, we calculated a majority rule consensus tree after omitting the first 25% of the trees as burn-in.

2.5 *Direct optimization analysis (dynamic homology)*

The direct optimization method was first proposed by Wheeler (1996) as an algorithm to process unaligned nucleotide sequences alone or in conjunction with morphological and aligned molecular data to search optimal topologies using maximum parsimony. Cladogram length during tree search is calculated by the sum of the costs for all hypothesized substitutions and insertion/deletion events (INDELs) via simultaneous evaluation of nucleic acid sequence homologies and cladograms (Wheeler et al. 2006). Throughout the analysis, for each examined topology, potentially unique schemes of positional homologies are dynamically postulated, tested by character congruence, and selected based on the overall minimal cost of character transformations. Detailed properties of the method and its relative advantages in comparison to conventional phylogenetic analysis have been extensively discussed elsewhere and will not be explored here (Wheeler 1996; Wheeler & Hayashi 1998; Phillips et al. 2000; Wheeler et al. 2001; but see Kjer et al. 2006, 2007).

Phylogenetic inference based on nucleotide sequences requires the assignment of specific numerical values for alignment and analysis parameters that define cost regimes for INDELs and transformations (e.g., transversion and transition costs), which can be expressed as cost ratios such as gap: transversion: transition. Because utilization of a single cost regime (traditionally used for phylogenetic studies based on molecular data) does not allow evaluation of how sensitive tree topologies are to any specific set of cost regimes, Wheeler (1995) suggested the selection of a number of parameter sets consisting of the combination of different values for each component of the cost regime (i.e., gap: transversion: transition) within his concept of sensitivity analysis. Sensitivity analysis identifies robust clades, which would be considered those present under most or all parameter sets, from more "unstable" clades resulting from one or a few cost regimes. Since different cost regimes can often generate conflicting topologies, character congruence among different data partitions can be used as an external criterion to choose among parameter sets (Wheeler 1995; Wheeler & Hayashi 1998; Schulmeister et al. 2002; Agesen et al. 2005). Using this criterion, the combination of parameter values that maximize character congruence (and hence minimize homoplasy inherent in a combined analysis) can be calculated with the incongruence length difference (ILD; Mickevich & Farris 1981; Farris et al. 1994).

Within this framework, in the present study, we submitted all data partitions to a simultaneous cladistic analysis using direct optimization as implemented in POY ver. 4.0 (Varon et al. 2007). We performed tree search using 7200 random addition sequences followed by branch swapping with simulated annealing algorithm (Kirkpatrick et al. 1983), keeping one best tree for each starting tree,

on a 24 x 3.2 GHz AMD64 CPU cluster. We used an array of 9 parameter sets to examine the stability of the phylogenetic hypotheses in relation to cost regimes for INDELs (gaps), transversions, and transitions. These parameters considered ranges of costs of 1 to 8 for gaps and 1 to 4 for transformations, resulting in the following cost ratios for gap: transversion: transition: 111, 112, 121, 211, 212, 221, 411, 412, and 421. To compute ILD values ($= \text{Length}_{\text{combined}} - (\text{Length}_{\text{MORPH}} + \text{Length}_{\text{DNA}}) / \text{Length}_{\text{combined}}$), we submitted the molecular partition to the same search protocol as described above, and analyzed the morphological matrix in TNT version 1.1 (Goloboff & Giannini 2008) with 1000 random additions and branch swapping by alternate SPR and TBR algorithms.

3 RESULTS

3.1 Comparisons of molecular and morphological data partitions

ILD tests indicated that morphological and molecular datasets were strongly congruent ($p = 0.99$). The majority of nodes had positive PBS values for both molecular (86% of nodes > 0) and morphological (73% of nodes > 0) data partitions, indicating both sets of characters contributed positively to resolution of the tree in the combined analysis. Relative to the molecular data, morphological data also had a greater percentage of phylogenetically informative (PI) characters (56% of morphological characters, versus 30% of the molecular character set). We calculated the relative support provided by molecular and morphological data partitions by summing the PBS values of all nodes ($\text{PBS}_{\text{DNA}} = 134.6$, $\text{PBS}_{\text{MORPH}} = 11.3$) and examining information content relative to the number of phylogenetically informative characters for each partition (e.g., Baker et al. 1998). Although morphological characters represented $< 4\%$ of the total character matrix, they had higher overall PBS values relative to the number of phylogenetically informative (PI) sites ($\text{PBS}_{\text{MORPH}} / \text{PBS}_{\text{DNA}} > \text{PI}_{\text{MORPH}} / \text{PI}_{\text{DNA}}$), suggesting the morphological data provided more support for nodes in the tree relative to the size of its character set.

3.2 Bayesian analysis

The Bayesian combined-analysis tree resolved several major groupings of taxa (Fig. 1). A clade including the Oregoniidae and the mithracid genus *Micippa* branched first (BPP = 81), and then a clade (with the majid species *Maja* branching at the base) consisting of the Epialtidae, Mithracidae, Pisidae, and the inachid genus *Stenorhynchus*. Within this latter grouping, there were well-supported clades of mithracid and tyichid genera (*Pitho*, *Microphrys*, and *Mithraculus*; BPP=100); two epialtid genera (*Acanthonyx* and *Menaethius*; BPP = 99); and a clade of epialtid and pisid taxa (*Taliepus*, *Pugettia*, *Herbstia*, and *Libinia*; BPP = 91). Members of Oregoniidae (*Chionoecetes* + *Hyas*, BPP = 100) and the family Pisidae (*Libinia* + *Herbstia*, BPP = 100) both formed monophyletic groups, but there was otherwise no support for monophyly of majoid families recognized by Ng et al. (2008), McLaughlin et al. (2005), or Clark & Webber (1991).

3.3 Direct optimization analysis

For direct optimization analyses, the set of alignment cost parameters that minimized homoplasy between datasets (i.e., had the lowest ILD value) corresponded to the 1:1:1 cost weighting scheme (gaps: transversions: transitions; ILD values not shown). To evaluate support for different nodes in this topology given different sets of cost parameters, we used the sensitivity plot to indicate the proportion of parameter sets supporting a given node. In this topology (Fig. 2), the Oregoniidae formed a monophyletic group branching at the base of the majoids, followed by the majid genus *Maja* (similar to the Bayesian tree). The mithracid genus *Micippa* branched at the base of the remaining majoids. In contrast to the Bayesian tree (where it grouped with the mithracid genera *Mithraculus* and *Microphrys*), the tyichid species *Pitho lherminieri* formed an idiosyncratic clade with the inachid

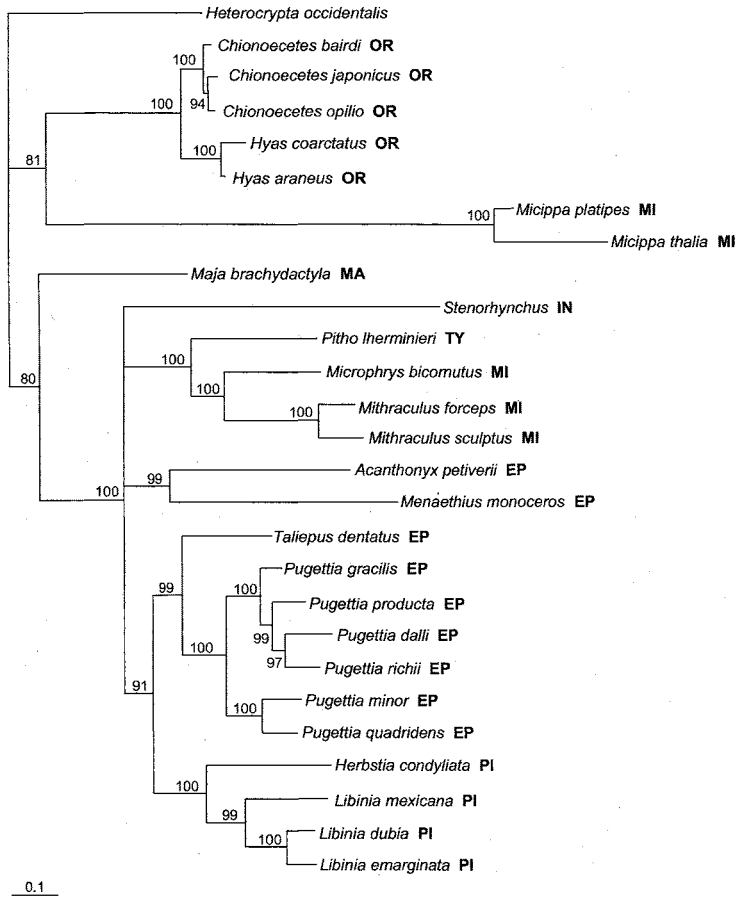


Figure 1. Bayesian tree of the Majoidea based on combined molecular and morphological partitions. Numbers by each node indicate Bayesian posterior probability values for that node. Abbreviations in bold after each species indicate family affiliations (after McLaughlin et al. 2005; OR = Oregoniidae, MI = Mithracidae, MA = Majidae, IN = Inachidae, TY = Tychidae, EP = Epialtidae, PI = Pisidae).

Stenorhynchus and the epialtid species *Menaethius monoceros* (Fig. 2). Remaining epialtid species formed a clade with the Pisidae. As in the Bayesian tree, there was support for monophyly only for the Oregoniidae and Pisidae families.

4 DISCUSSION

In this study, we found that molecular and larval morphology data were strongly congruent, with both partitions independently contributing positively to the support of most relationships. Given the increasing availability of DNA sequence data, the utility of morphological data in phylogenetic inference is often debated (Scotland et al. 2003; Jenner 2004; Lee 2004), in part because many combined-analysis studies show significant incongruence between relationships inferred from morphological and molecular character sets and/or an insignificant contribution of morphological data to tree topology (Baker et al. 1998; Wortley & Scotland 2006). Indeed, previous studies have shown relationships among the majoids inferred from molecular data (Hultgren & Stachowicz in press) are incongruent with familial relationships inferred from adult morphology, even with the most recent reclassifications of majoid families (e.g., Ng et al. 2008). The high levels of congruence between

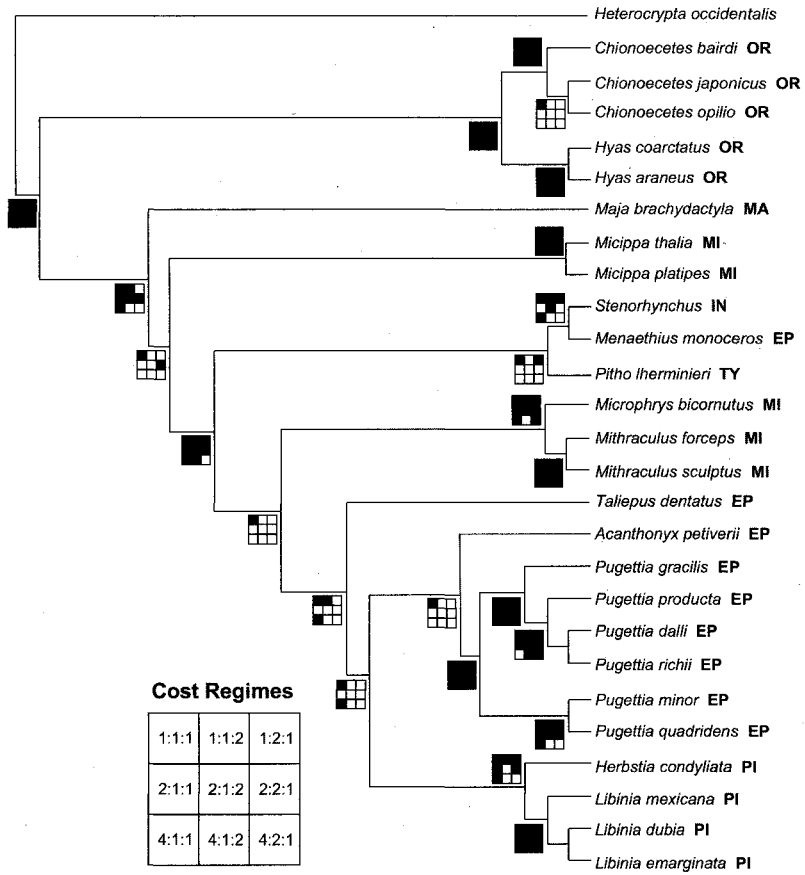


Figure 2. Most congruent phylogenetic hypothesis based on direct optimization of molecular and morphological data for Majoidea assuming cost ratios of 1:1:1 (gap: transversion; transition ratios). Sets of boxes below each node indicate the sensitivity plots for which dark fields indicate those parameter sets in which the respective group came out as monophyletic. The order of parameter sets is represented in the box at the bottom left of the figure. Abbreviations in bold after each species indicate family affiliations (abbreviations as in Figure 1).

molecular and larval morphology datasets in this study suggest that for the majoids, molecular and larval characters may provide more phylogenetic information than the adult morphological characters used to place majoids into families, although no phylogeny based on adult morphology has been published to date. That one source of morphological data should be more congruent than another with regards to relationships proposed by molecular data supports earlier observations made by decapod workers that adult morphological characters are often more convergent than larval characters (Williamson 1982). This result also suggests that any decisions to include additional morphological data in a particular study should involve investigation of whether characters in question are under strong selection that might obscure branching patterns (e.g., convergence of similar adult body morphologies due to selection patterns rather than homology). The difficulty of defining morphological characters and making accurate assessments of primary homology (*sensu de Pinna 1991*) often limits the number of characters in these datasets, relative to obtaining sequence data (Baker et al. 1998; Scotland et al. 2003; Wahlberg et al. 2005; Wortley & Scotland 2006). However, morphological characters will always represent a unique set of characters that is independent of sequence data, unlike, for example, a “multi-locus” dataset consisting of two different mitochondrial loci.

Additionally, morphological characters often exhibit less homoplasy and a higher proportion of phylogenetically informative characters than molecular data (Lee 2004) and can often resolve different (but complementary) portions of the tree from molecular data (Jenner 2004), suggesting that combining these multiple types of data may contribute positively to phylogenetic reconstruction (Baker et al. 1998; Ahyong & O'Meally 2004; Wahlberg et al. 2005).

Although trees constructed with direct optimization vs. Bayesian methods reconstructed similar relationships at many of the apical nodes in our study, branching patterns of deeper nodes appear to be sensitive to sequence alignment and inclusion or exclusion of insertion/deletion events (INDELs). For example, the idiosyncratic mithracid genus *Micippa* grouped with the Oregoniidae at the base of the Bayesian tree but branched in a different region in the direct optimization tree. This pattern may not be surprising, given that > 60% of the molecular data consisted of ribosomal gene sequences (16S and 28S) in which INDELs may make multiple alignment problematic. However, it is difficult to compare the effects of different alignment methods and INDEL inclusion independently of differences in phylogenetic inference methods, e.g., model-based methods (utilized in the Bayesian tree) versus maximum parsimony (utilized in the direct-optimization tree). Additionally, support for certain clades in the majoid combined analyses is difficult to directly compare between the topology produced by direct optimization, in which clade stability was assessed using sensitivity plots for a particular node, and trees produced by Bayesian analysis, in which support for a certain clade was assessed by posterior probability.

Despite differences in deep branching patterns due to differences in alignment, inclusion or exclusion of INDELs, and optimality criteria, some groupings were supported in multiple forms of analysis. One such grouping was a monophyletic Oregoniidae branching at the base of the tree. Although previous molecular phylogenies also supported a monophyletic Oregoniidae, they did not conclusively resolve the position of this clade relative to the remaining majoids (Hultgren & Stachowicz in press). Utilization of a combined molecular and morphological dataset in this study strongly supports the Oregoniidae as the most basally branching majoid family, as has been proposed in earlier studies of this group (Rice 1983, 1988; Clark & Webber 1991; Marques & Pohle 1998). Unlike the majority of majoid families, which contain species distributed worldwide, all members of the Oregoniidae are primarily limited to boreal regions (Griffin & Tranter 1986), and similarity in geographic range and/or habitat may help explain why this family is the only group unambiguously resolved in analyses of larval morphology, molecular data, and adult morphology. Although the two pisid genera represented in this study (*Herbstia* and *Libinia*) were monophyletic, molecular and morphological studies with higher taxon sampling (Marques & Pohle 2003; Hultgren & Stachowicz in press) find no support for the monophyly of the Pisidae. The Mithracidae were paraphyletic in all trees in this study, primarily because the genus *Micippa* never grouped with the remaining mithracids. Placement of *Micippa* relative to the remaining majoids was generally unstable (as has been noted in other studies, e.g., Hultgren & Stachowicz in press) and sensitive to different alignment and tree construction methods (Figs. 1, 2). There was likewise no support for the Majidae family *sensu* Ng et al. (2008) (Mithracinae + Majinae). The family Epialtidae was paraphyletic in this study, though in both Bayesian and direct optimization trees there was a close phylogenetic alliance between selected members of the Epialtidae and Pisidae. In this case, the recent Ng et al. (2008) reclassification of the Epialtinae and Pisinae (i.e., Epialtidae and Pisidae) as subfamilies within a larger family (Epialtidae *sensu* Ng et al. 2008) is supported; close relationships between the Pisidae and Epialtidae also were noted in some of the earliest systematic investigations of majoid relationships and larval morphology (Rice 1980, 1988).

The difficulty of using adult morphological characters to establish different family groupings within the Majoidea is reflected in frequent reclassification of majoid families (Griffin & Tranter 1986; Clark & Webber 1991; Martin & Davis 2001; McLaughlin et al. 2005; Ng et al. 2008) and in the failure of subsequent molecular and larval morphology phylogenies to support monophyly of most of these families. However, molecular and larval morphology data in this study both supported

a few key taxonomic groupings in combined-analysis Bayesian and direct optimization trees. Both trees supported a monophyletic Oregoniidae branching near the base of the tree, confirming earlier studies suggesting this group represents one of the oldest majoid lineages (Rice 1980, 1988; Clark & Webber 1991; Marques & Pohle 1998). Our study also suggests at least two distinct groupings of the Mithracidae, namely one (*Mithraculus* + *Microphrys*) that may be related to the tyichid species *Pitho lherminieri* and one (the mithracid genus *Micippa*) more distantly related to the remaining mithracids. Sampling molecular and morphological characters from additional taxa, especially from hyper diverse regions underrepresented in our study (such as the Indo-Pacific), is warranted to further examine these hypothesized groupings.

We would like to emphasize that the relationships suggested herein represent tentative hypotheses based on the data at hand, namely, <10% of the 170+ majoid genera in the world. Additional focus on the Inachidae, Majidae, and Inachoididae (the latter of which was not sampled in this study) is crucial to further resolve branching patterns at the base of the majoid tree. Rigorous testing of the monophyly of the Majoidea—namely, whether it includes the Lambrachaeidae, Paratymolidae, and Hymenosomatidae (Guinot & Richer de Forges 1997; Števcíć 2005; Ng et al. 2008)—is also important in order to properly describe the higher-level systematics of this group. However, the positive contribution of both molecules and morphology to resolution of relationships within the majoids suggests that combining these different sources of data may hold strong potential for researchers to establish a more stable classification of majoid families in the future.

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APPENDIX 1

Larval morphology character matrix for taxa in the study. A “?” indicates missing data for that character; parentheses surround characters ambiguous for two states.

| Family | Species | Character matrix | Reference |
|-------------|--|--|-----------------------------------|
| Epiplatidae | <i>Acanthonyx petiverii</i> (Milne-Edwards, 1834) | 0100121020212110110200011001100210001???01?43412????? | Hiyodo et al. 1994 |
| | <i>Menaethius monoceros</i> (Latreille, 1825) | 0110121101021211111020111011101210001???????????????? | Gohar & Al-Kholy 1957 |
| | <i>Pugettia quadridens</i> (de Haan, 1850) | 0100120201011121111020001200110021000101001?24302113?0 | Kornienko & Korn 2004 |
| | <i>Talipeus dentatus</i> (Milne-Edwards) | 0100120111021221111020001001100210001???11?31312????? | Fagetti & Campodonico 1971 |
| | <i>Stenorhynchus seticornis</i> (Herbst, 1788) | 11001212010112111110200021111102101110?014?25202?01? | Yang 1976, Paula & Cartaxana 1991 |
| Majidae | <i>Maja brachydactyla</i> (Balss, 1922) | 000011000110121111102000111110001000111012?4310201000 | Clark 1986 |
| | <i>Micippa thalia</i> (Herbst, 1803) | 0110120011121201011020001011100010001???????????????? | Kurata 1969 |
| Mithracidae | <i>Micippa platipes</i> (Ruppell, 1830) | 0110120011121201011020001011100010001???????????????? | Siddiqui 1996 |
| | <i>Microphrys bicornutus</i> (Latreille, 1825) | 010012000102121101101001101110021000101011?6?30101010 | Gore et al. 1982 |
| | <i>Mithraculus forceps</i> (Milne-Edwards, 1875) | 0100120001(01)212110110100110111002100010001106231001001 | Wilson et al. 1979 |
| | <i>Mithraculus sculptus</i> (Lamarck, 1818) | 0100120001(01)212110110100110111002100010001106231001001 | Rhyme et al. 2006 |
| | <i>Chionoecetes japonicus</i> (Rathbun, 1924) | 000000010(12)5010100001000200000010000000003?6?11????? | Motoh 1976 |
| Oregoniidae | <i>Hyas coarctatus alutaceus</i> Brandt, 1851 | 00000000101501010000100020000001000000000?41212????? | Christiansen 1973, Pohle 1991 |
| | <i>Hyas araeus</i> (Linnaeus, 1758) | 00000000101501010000100020000001000000000?41212????? | Christiansen 1973, Pohle 1991 |
| | <i>Herbstia condyliata</i> (Fabricius, 1787) | 010012001102121101102000000010021000101001?4130100200 | Guerao et al. 2008 |
| Pisidae | <i>Libinia dubia</i> (H. Milne Edwards, 1834) | 010012011101101101102001100110021000100011?4(0)36011110 | Sandifer & Van Engel 1971 |
| | <i>Libinia emarginata</i> (Leach, 1815) | 010012011101101101102001???110021000101001?4130011?1? | Johns & Lang 1977 |
| | <i>Pitho therminieri</i> (Schramm, 1867) | ?10012011102121101?010011011100210011???????????????? | Marques et al. unpublished data |

APPENDIX 2

Morphological characters of majoid larvae used in the analyses.

1. Zoal rostral spine: present (0), absent (1).
2. Zoal lateral spines: present (0), absent (1).
3. Zoal dorsal spine: present (0), absent (1).
4. Zoal carapace serrulation: ornamentation absent (0), ornamentation present (1).
5. Zoa II subterminal setation on the antennule: present (0), absent (1).
6. Zoal exopod morphology of the antenna: terminal spine minute, less than half length of apical setae (0), terminal spine half or more length of apical setae but not extending beyond tip of setae (1), terminal spine extending beyond tip of setae, latter inserted distally to proximal half of shaft (2), terminal spine extending much beyond setae, latter inserted on proximal half of shaft (3).
7. Proximal segment of the zoal maxillulary endopod: seta present (0), seta absent (1).
8. Distal segment of the zoal maxillulary endopod: six setae (0), 5 setae (1), 4 setae (2), 3 setae (3).
9. Ontogenetic setal transformation of the maxillulary coxa from zoea I to zoea II: stasis at 7 additional 8th seta (0), additional 9th seta (1).
10. Zoal proximal setation of maxillulary basis: plumodenticulate (0), pappose (1).
11. Ontogenetic setal transformation of the maxillulary basis from ZI to ZII: 7 to 10 (0), 7 to 9 (1), 7 to 8 (2).
12. Ontogenetic setal transformation of the proximal lobe of the maxillary coxa from ZI to ZII: stasis at 3 (0), stasis at 4 (1), stasis at 5 (2), 3 to 4 (3), 4 to 5 (4), stasis at 4 (5).
13. Ontogenetic setal transformation of the proximal lobe of the maxillary basis from ZI to ZII: 5 to 6 (0), stasis at 5 (1).
14. Ontogenetic setal transformation of the distal lobe of the maxillary basis from ZI to ZII: stasis at 4 (0), 5 to 6 (1), 4 to 5 (2).
15. Zoal setation of the maxillary endopod: 6 setae (0), 5 setae (1), 4 setae (2), 3 setae (3).
16. Lobes of the zoal maxillary endopod: bilobed (0), single lobed (1).
17. Setation on the zoal basis maxilliped 1: 10 setae (0), 9 setae (1), 11 setae (2).
18. Setation on the zoal basis of maxilliped 2: 4 setae (0), 3 setae (1), 2 setae (2), 1 setae (3), absent (4).
19. Setation on the proximal zoal endopod segment of maxilliped 2: seta present (0), seta absent (1).
20. Setation on the penultimate segment of the zoal endopod of maxilliped 2: seta present (0), seta absent (1).
21. Setation on the distal segment of the zoal endopod of maxilliped 2: 6 setae (0), 5 setae (1), 4 setae (2), 3 setae (3).
22. Relative length of terminal setae on the distal segment of the zoal endopod of maxilliped 2: one shorter (0), same length (1).
23. Spine on the distal segment of the zoal endopod of maxilliped 2: present (1), absent (0).
24. Dorsal lateral process on the third zoal abdominal somite: present (0), absent (1).
25. Middorsal setae on the first abdominal somite in zoea II: 5 setae (0), 3 setae (1), 2 setae (2), absent (3).
26. Middorsal setae on the second abdominal somite in zoea II: present (0), absent (1).
27. Middorsal setae on the third abdominal somite in zoea II: present (0), absent (1).
28. Middorsal setae on the fourth abdominal somite in zoea II: present (0), absent (1).
29. Middorsal setae on the fifth abdominal somite in zoea II: present (0), absent (1).
30. Zoal acicular process on the second abdominal somite: present (1), absent (0).
31. 6th somite in zoea II: differentiated (0), not differentiated (1).
32. Zoal telson furcal spination: 3 spines (0), 2 spines (1), 1 spine (2), no spine (3).
33. Zoal II telson furcal arch setation: 8 setae (0), 6 setae (1).
34. Megalopa uropods (pleopods on the 6th abdominal somite): present (0), absent (1).
35. Pronounced antennal exopod process in megalopa: present (1), absent (0).
36. Fusion of megalopa antennal flagellar articles 2+3: present (1), absent (0).
37. Fusion of megalopa antennal flagellar articles 4+5: present (1), absent (0).
38. Seta on the first segment of the peduncle of the antennule: present (1), absent (0).
39. Seta on the second segment of the peduncle of the antennule: 2 setae (0), 1 seta (1), absent (2).
40. Seta on the third segment of the peduncle of the antennule: 1 seta (0), 2 setae (1).
41. Setae on the distal segment of the antenna: 4 setae (0), 3 setae (1).
42. Setation of the palp of the mandible: 8 setae (0), 5 setae (1), 4 setae (2), 11 setae (3), 6 setae (4), 1 seta (5).

43. Epipod setae on the maxillule: present (1), absent (0).
44. Setation on the endopod of the maxillule: 6 setae (0), 5 setae (1), 4 setae (2), 3 setae (3), 2 setae (4), 1 seta (5), seta absent (6).
45. Ontogenetic change from zoea II to megalopa on the coxal endite of the maxillule: 8 to 11 (0), 8 to 10 (1), 7 to 10 (2), 7 to 11 (3), 7 to 9 (4), 7 to 8 (5), stasis to 7 (6).
46. Ontogenetic change from zoea II to megalopa in the distal lobe of the coxal endite of the maxilla: 4 to 6 (0), 4 to 5 (1), stasis at 4 (2), stasis at 3 (3).
47. Seta on the proximal segment of the exopod on the third maxilliped: present (1), absent (0).
48. Setation on the distal segment on the exopod of the third maxilliped: 6 setae (0), 5 setae (1), 4 setae (2).
49. Setation on the second abdominal somite: 8 setae (0), 6 setae (1): 2 setae (3).
50. Setation on the third abdominal somite: 8 setae (0), 6 setae (1), 2 setae (3).
51. Setation on the fourth abdominal somite: 8 setae (0), 6 setae (1), 10 setae (2), 4 setae (3).
52. Setation on the fifth abdominal somite: 8 setae (0), 6 setae (1).
53. Setation on the sixth abdominal somite: 2 setae (0), none (1).

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